ASSESSMENT OF DIATOM COMPOSITION AT SEVERAL LOCATIONS ALONG TEBRAU STRAITS FOR ASSIGNING SCENE OF DROWNING

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ABSTRACT

The use of diatoms for investigation of drowning death cases has been suggested in the literature. This study designates the assemblages of diatoms at different stations along the Tebrau Straits to establish diatomological mapping as a forensic measure to determine the site of drowning based on association of diatom assemblages recovered from the organ samples with that of the Straits. In each station, water samples from 3 substations (200 m from the shore), separated by a distance of 500 m apart were collected at 2 m depth using a Horizontal Van Dorn sampler. Replicating the same procedure, water samples from 5 different stations distanced by about 20 km from one another, along the Johor Straits were sampled during March-April 2015. To study the recoverability of diatoms from 13 real forensic cases, ranging between 9 months – 4 years, samples of organs (livers, lungs and kidneys) supplied by the pathologist were analyzed. The samples were processed using acid digestion technique and taxonomic identification of diatoms with their percentage assemblages were analyzed under light microscope. A total of 26 genera found from seawater samples were identified. The percentages assemblage of diatoms varied among the different sampling stations and hence, may be useful for distinguishing the stations. While diatoms remained recoverable in lungs and kidneys in majority of suspected drowning cases, the same was not observed in livers. The fact that diatoms were not observed in 3 later (11-12 months) cases, inferring the cause of death due to drowning may prove inconclusive.

ABSTRAK

Penggunaan diatom untuk penyiasatan kes mati lemas telah dicadangkan dalam ulasan tulisan. Kajian ini meninjau himpunan diatom pada stesen yang berbeza sepanjang Selat Tebrau untuk menghasilkan pemetaan diatomologikal sebagai satu langkah forensik bagi menentukan tapak mati lemas berdasarkan perkaitan diatom yang ditemui daripada sampel organ dan Selat Tebrau. Dalam setiap stesen, sampel air daripada 3 substesen (200 m dari pesisiran pantai), berasingan jarak 500 m telah dikumpul pada kedalaman 2 m menggunakan 'Horizontal Van Dorn sampler'. Sampel air daripada 5 stesen berbeza dijarakkan oleh 20 km dari satu sama lain, sepanjang Selat Tebrau telah disampel pada March-April 2015 dengan mengulangi langkah yang sama. Untuk mengkaji kebolehpulihan diatom dari 13 kes forensik sebenar, berjulat antara 9 bulan sehingga 4 tahun, sampel organ (hati, paru-paru dan buah pinggang) diperoleh daripada ahli patologi telah dianalisis. Sampel-sampel berikut diproses menggunakan teknik cernaan asid dan pengenalan taksonomi diatom serta peratusan himpunan diatom dianalisis menggunakan mikroskop cahaya. Sejumlah 26 genera daripada sampel air laut telah dikenalpasti. Peratus himpunan diatom yang berbeza di setiap stesen persampelan, membolehkan perbezaan stesen sepanjang Selat Tebrau dilakukan. Diatom yang terdapat dalam paru-paru dan buah pinggang dalam majoriti kes yang disyaki mati lemas tetap dibaikpulih, walaubagaimanapun, perkara yang sama tidak dapat diperhatikan pada diatom yang terdapat dalam hati. Diatom tidak diperhatikan dalam 3 kes (11-12 bulan) kemudian, membuktikan punca kematian adalah disebabkan oleh mati lemas mungkin tidak tepat.

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LIST OF ABBREVIATION

CO_2	Carbon dioxide
Cu	Copper
DOE	Department of energy
E	East
FAAS	Flameless atomic absorption spectrometry
FC	Fecal coliform
Fe	Iron
FS	Fecal streptococci
g	Gram
ICP-MS	Inductively coupled plasma mass spectrometry
km	Kilometre
L	Litre
LV	Left ventricle
m	Metre
min	Minute
mL	Millilitres
mm	Millimetres
M	Molar
N	North
NaCl	Sodium Chloride
pH	Potential hydrogen
Pb	Lead
RV	Right ventricle
rRNA	Ribosomal ribonucleic acid
rpm	Revolution per minute

$Si(OH)_4$	Silicic acic
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscope
sp.	Species
Sr	Strontium
TH	Tyrosine hyroxylase
USA	United States of America
µm	Micrometer
µL	Microlitre
Zn	Zinc

SYMBOLS

°C	Degree Celcius
%	Percent

CHAPTER 1

INTRODUCTION

1.1 Background of study

In medico-legal investigation of bodies recovered from water, the aim of the investigations often focuses on determination of the cause and manner of death along with the site of death. Deaths in water may be due to drowning, injuries, intoxications or natural causes and, in this context diatoms in airways as well as organs are widely studied (Lunetta and Modell, 2005). Diatom is a microscopic unicellular aquatic algae belonging to *Bacillariophyta* (Pollanen, 1997) and first described in drowning victim at the end of 19th century (Revenstof, 1904). Since then, diatom test has been used as forensic evidence in cases involving bodies recovered from water (Lunetta and Modell, 2005).

Diatom test is considered as the gold standard in diagnosing ante-mortem or post-mortem drownings and assigning drowning site especially when dealing with bodies recovered from water (Piette, 2006). In general, ante-mortem drowning caused diatoms to reach lungs *via* water aspiration, prior to being distributed to other organs through bloodstream. On the contrary, post-mortem drowning caused diatoms to passively enter airways and not travelling to the other organs due to lack

of circulation system after death (Lunetta and Modell, 2005). Comparing the diatoms found in organ sample from drowned bodies with diatoms recovered from water samples can also suggest the drowning site (Vinayak *et al.,* 2013). The test required complete destruction of other particles than diatoms (such as organ and debris), excluding the diatom frustules (Lunetta and Modell, 2005). Ming *et al.* (2007) indicated the common extraction techniques applied in diatom test are digestion using (1) strong acid (hydrochloric acid, nitric acid), (2) using solubilizers (soluene-350) and (3) enzymatic digestion (Proteinase K).

1.2 Problem Statement

Diatoms are location specific, attributable to variations in salinity, temperature and presence of organic matters (Cameron, 2004), and hence, may prove useful in assigning the site of drowning. However, due to unavailability forensic baseline data within Johor marine ecology, investigation concerning site of drowning can be difficult. Hence, it is pertinent to conduct this study for exploring the diatom distributions and its mapping, specifically within the bustling waters of the Tebrau Straits, in view of its application in assigning drowning and suggesting the possible localities.

In Malaysia, forensic pathologists always rely on histopathological findings to draw conclusions pertaining to deaths due to drowning and, utilization of diatoms in this aspect remains unreported. This may be due to the lack of information and understanding on its recoverability in human organs, limiting its application in forensic cases. Considering such limitation, the specific attempts made in this present study to recover diatoms in such organs as lungs, kidneys and livers using the acidic digestion method, merits consideration.

1.3 Objectives

This study specifically designed:

(a) To establish diatomological mapping related to the findings of diatoms assemblages found at several locations along the Tebrau Straits.

(b) To study the recoverability of diatoms from organ samples collected from suspected drowning cases using acid-digestion method.

1.4 Scope of Study

This study involved the collection of seawater samples from Lebam River, Kabong Village, Johor Causeway, Pendas and Tanjung Piai of the Tebrau Straits. In addition, organ samples from bodies, presumably died because of drowning during December 2012 and April 2015, collected by a forensic pathologist at Hospital Sultanah Aminah Johor Bahru were also analyzed. Extraction of diatoms from water and organ samples was made using the acid digestion method suggested by the previous researchers (Yen and Jayaprakash, 2007; Malik and Kaidan, 2013). Upon identifying the genera of diatoms following the identification keys provided by previous researchers (Shamsudin, 1990, 1991; Spaulding *et al.*, 2010), qualitative and quantitative assessments were made.

1.5 Significance of Study

In addition to providing the diatomological mapping for the Tebrau Straits, the data reported here may be useful in real forensic cases for indicating the site of drowning, whenever specific assemblages of diatoms are recovered from the different organs of victims, purportedly drowned at locations along the Straits. Such aspect acquires forensic significance for suggesting the primary crime scenes, an important element for establishing the fact of the case as well as disputing an alibi.

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